

FIG. I

Heller Ehrman White & McAuliffe LLP
Sheet 2 of 5
ARTIFICIAL CHROMOSOMES, USES THEREOF AND METHODS
FOR PREPARING ARTIFICIAL CHROMOSOMES
DOCKET NO. 24601-4021
Filed: April 17, 2001

حاديا أغيما الجالجيات الرسيد

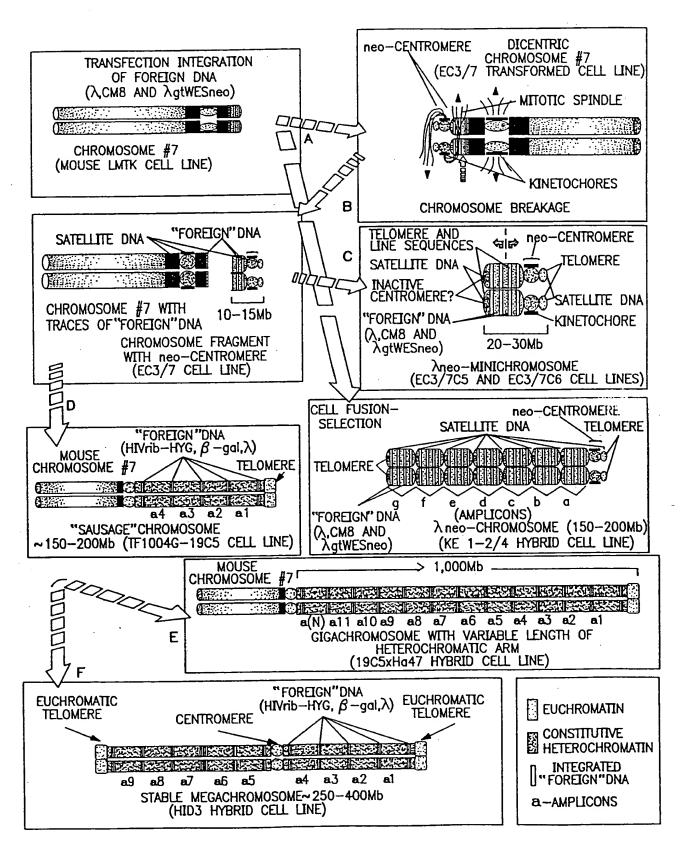


FIG. 2

Heller Ehrman White & McAuliffe LLP
Sheet 3 of 5
ARTIFICIAL CHROMOSOMES, USES THEREOF AND METHODS
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PRIMARY REPLICATION INITIATION SITE (MEGAREPLICATOR)

SECONDARY ORIGINS OF REPLICATION

MEGAREPLICON OF THE CENTROMERIC REGION OF MOUSE CHROMOSOMES WITH TWO~7.5Mb TANDEM BLOCKS OF MOUSE MAJOR SATELLITE DNA (mSAT) FLANKED BY NON-SATELLITE DNA SEQUENCES

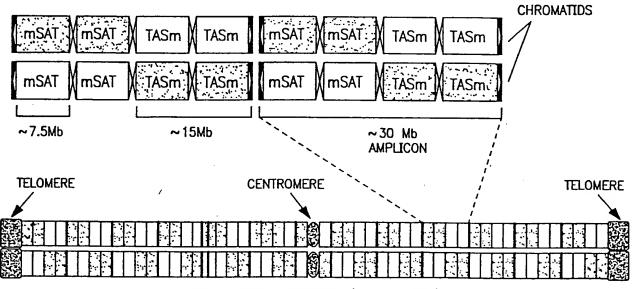
INTEGRATION OF "FOREIGN" DNA (pH132, pCH110, \(\lambda\))

mSAT mSAT

REPLICATION ERROR GENERATES INVERTED MEGAREPLICONS

mSAT (mSAT () TASm () TASm

AMPLIFICATION PRODUCES A TANDEM ARRAY OF IDENTICAL CHROMOSOME SEGMENTS (AMPLICONS) THAT CONTAIN TWO INVERTED MEGAREPLICONS BORDERED BY THE HETEROLOGOUS ("FOREIGN") DNA



STABLE MEGACHROMOSOME (~250-400Mb)

FIG. 3

T)

T)

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EC3/7

MOUSE LMTK" (FIBROBLAST CELL LINE WITH neo-CENTROMERE)

SINGLE-CELL SUBCLONING

FUSION WITH CHO K20 CELLS AND ▼ SELECTION WITH G418 AND HAT

EC3/7C5

MOUSE LMTK FIBROBLAST CELL LINE WITH THE neo-MINICHROMOSOME AND THE FORMERLY DICENTRIC CHROMOSOME

KE1 - 2/4

MOUSE-HAMSTER HYBRID CELL LINE WITH THE STABLE \(\lambda\) neo-CHROMOSOME

COTRANSFECTION WITH PLASMIDS pH132 (ANTI-HIV RIBOZYME AND HYGROMYCIN-RESISTANCE ĠENES), pCH110 (lacZ GENE) AND λcl 875 Sam7 (λPHAGE), SELECTION WITH HYGROMYCIN B

TF1004G-19C5

MOUSE LMTK FIBROBLAST CELL LINE WITH neo-MINICHROMSOME AND STABLE SAUSAGE CHROMOSOME

FUSION WITH CHINESE HAMSTER OVARY CELLS (CHO K20 CELL LINE), SELECTION WITH HAT AND HYGROMYCIN B.

19C5xHa4, 19C5xHa3 | → RECLONING → 19C5xHa47 (CARRIES THE GIGACHROMSOME)

MOUSE-HAMSTER HYBRID CELL LINES CARRYING THE neo-MINICHROMOSOME AND THE SAUSAGE CHROMOSOME AND COUNTAINING A COMPLETE HAMSTER GENOME AND PARTIAL MOUSE GENOME

BrdU TREATMENT, SINGLE-CELL CLONING,

SELECTION WITH ▼HYGROMYCIN B

H1D3

BrdU TREATMENT, SINGLE-CELL CLONING. SELECTION WITH G418, BrdU

TREATMENT AND RECLONING

MOUSE-HAMSTER HYBRID CELL LINE CARRYING A

MEGACHROMOSOME BUT NO MINICHROMOSOME

FUSION WITH CD4+ HeLa CELLS CONTAINING neor, SELECTION WITH **G418 AND**

H1xHe41

HYGROMYCIN B

MOUSE-HAMSTER-HUMAN HYBRID CELL LINE CARRYING THE MEGACHROMOSOME AND A SINGLE HUMAN CHROMOSOME WITH CD4 AND

G3D5

G3D6

MOUSE-HAMSTER HYBRID CELL LINES CARRYING: neo- MINICHROMOSOME ONLY

MEGACHROMOSOME AND neo-MINICHROMOSOME

RECLONE AND GROW IN G418 AND HYGROMYCIN B

GHB42

CARRIES MEGA-CHROMOSOME AND neo-MINICHROMOSOME **GB43**

IN G418

CARRIES neo-

MINICHROMOSOME ONLY

RECLONE AND GROW

neor GENES; CONTAINS COMPLETE HAMSTER AND PARTIAL MOUSE GENOMES

FIG. 4

